

**The extracellular loop of Man-PTS subunit IID is responsible for the sensitivity of
Lactococcus garvieae to garvicins A, B and C**

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SUPPLEMENTARY MATERIALS

Table S1. Bacterial strains, plasmids and primers used in this study

Strains, plasmids, primers	Description	Source (reference)
Strains		
<i>Bacillus cereus</i> IBB3390	indicator strain	IBB PAS
<i>Bacillus subtilis</i> BSB1	indicator strain	52
<i>Campylobacter jejuni</i> 12	indicator strain	53
<i>Campylobacter jejuni</i> 480	indicator strain	54
<i>Campylobacter jejuni</i> 81176	indicator strain	55
<i>Campylobacter coli</i> 23/1	indicator strain	53
<i>Candida albicans</i> CAI-4	indicator strain,	56
<i>Carnobacterium maltaromaticum</i> IBB3447	indicator strain	IBB PAS
<i>Enterococcus durans</i> IBB3441	indicator strain	IBB PAS
<i>Enterococcus faecalis</i> IBB3439	indicator strain	IBB PAS
<i>Enterococcus faecalis</i> IBB3444	indicator strain	IBB PAS
<i>Enterococcus faecalis</i> LMGT 2003	indicator strain	LMGT NMBU
<i>Enterococcus faecium</i> LMGT 2783	indicator strain	LMGT NMBU
<i>Enterococcus faecium</i> LMGT 2787	indicator strain	LMGT NMBU
<i>Escherichia coli</i> EC1000	host strain, Km ^r , <i>repA</i> ⁺ derivative of MC1000, carrying a single copy of the pWV01 <i>repA</i> gene in <i>glgB</i>	57
<i>Escherichia coli</i> TG1	host strain, Δ (<i>hsdMS-mcrB</i>) 5 Δ (<i>lac-proAB</i>) <i>supE thi-1 F'</i> (<i>traD36 proAB⁺lacI^qZAM15</i>)	58
<i>Lactobacillus casei</i> IBB3418	indicator strain	IBB PAS
<i>Lactobacillus casei</i> IBB3427	indicator strain	IBB PAS
<i>Lactobacillus casei</i> LOCK 0919	indicator strain	LOCK ⁵⁹
<i>Lactobacillus casei/paracasei</i> IBB3423	indicator strain	IBB PAS
<i>Lactobacillus casei/paracasei</i> IBB3425	indicator strain	IBB PAS
<i>Lactobacillus casei/paracasei</i> IBB3426	indicator strain	IBB PAS
<i>Lactobacillus casei/paracasei</i> IBB3428	indicator strain	IBB PAS
<i>Lactobacillus paracasei</i> IBB3424	indicator strain	IBB PAS
<i>Lactobacillus johnsonii</i> IBB3155	indicator strain	IBB PAS
<i>Lactobacillus kunkei</i> AH1	indicator strain	LMGT NMBU
<i>Lactobacillus kunkei</i> AH38	indicator strain	LMGT NMBU
<i>Lactobacillus kunkei</i> AH119	indicator strain	LMGT NMBU
<i>Lactobacillus paraplantarum</i> IBB3438	indicator strain	IBB PAS
<i>Lactobacillus plantarum</i> NC8	indicator strain	LMGT NMBU ⁶⁰
<i>Lactobacillus plantarum</i> WCSF1	indicator strain	LMGT NMBU ⁶¹
<i>Lactobacillus plantarum</i> IBB3036	indicator strain	IBB PAS
<i>Lactobacillus plantarum</i> IBB3433	indicator strain	IBB PAS
<i>Lactobacillus plantarum</i> IBB3436	indicator strain	IBB PAS
<i>Lactobacillus plantarum</i> subsp. <i>plantarum</i> IBB3434	indicator strain	IBB PAS
<i>Lactobacillus rhamnosus</i> IBB3429	indicator strain	IBB PAS

<i>Lactobacillus rhamnosus</i> LOCK 0900	indicator strain	LOCK ⁶²
<i>Lactobacillus rhamnosus</i> LOCK 0908	indicator strain	LOCK ⁶³
<i>Lactobacillus rhamnosus</i> GG	indicator strain	Dicoflor ⁶⁴
<i>Lactobacillus salivarius</i> IBB3154	indicator strain	IBB PAS ⁶⁵
<i>Lactococcus garvieae</i> IBB3403	indicator strain	IBB PAS
<i>Lactococcus garvieae</i> IBB66	indicator strain	IBB PAS
<i>Lactococcus lactis</i> IBB3404	indicator strain	IBB PAS
<i>Lactococcus lactis</i> IBB3411	indicator strain	IBB PAS
<i>Lactococcus lactis</i> NZ9000	indicator strain, <i>pepN::nisRnisK</i> integrated into chromosome	MoBiTec ⁶⁶
<i>Lactococcus lactis</i> QU5 LMGT 3419	indicator strain	LMBT NMBU
<i>Lactococcus lactis</i> subsp. <i>cremoris</i> IBB3409	indicator strain	IBB PAS
<i>Lactococcus lactis</i> subsp. <i>lactis</i> IL1403	indicator strain, host strain	INRA ⁶⁷
<i>Lactococcus lactis</i> subsp. <i>lactis</i> IBB2955	indicator strain	IBB PAS
<i>Lactococcus lactis</i> subsp. <i>lactis</i> IBB3407	indicator strain	IBB PAS
<i>Lactococcus raffinolactis</i> IBB91	indicator strain	IBB PAS
<i>Leuconostoc lactis</i> IBB3446	indicator strain	IBB PAS
<i>Leuconostoc mesenteroides</i> IBB3442	indicator strain	IBB PAS
<i>Leuconostoc mesenteroides</i> IBB3443	indicator strain	IBB PAS
<i>Listeria monocytogenes</i> EGD-e LMGT 2604	indicator strain	LMGT NMBU ⁶⁸
<i>Pediococcus acidilacti</i> LMGT 2002	indicator strain	LMGT NMBU
<i>Pediococcus parvulus</i> IBB3448	indicator strain	IBB PAS
<i>Pediococcus pentosaceus</i> IBB3369	indicator strain	IBB PAS
<i>Pseudomonas aeruginosa</i> ATCC 9027	indicator strain	ATCC
<i>Salmonella typhimurium</i> TT622	indicator strain	⁶⁹
<i>Staphylococcus aureus</i> ATCC 6538	indicator strain	ATCC
<i>Staphylococcus caprae</i> DSM-20608	indicator strain	DSMZ
<i>Staphylococcus delphini</i> DSM-20771	indicator strain	DSMZ
<i>Staphylococcus epidermidis</i> DSM-20044	indicator strain	DSMZ
<i>Staphylococcus hyicus</i> subsp. <i>chromogenes</i> DSM-20454	indicator strain	DSMZ
<i>Staphylococcus intermedius</i> DSM-20373	indicator strain	DSMZ
<i>Staphylococcus lugdunesis</i> DSM-4804	indicator strain	DSMZ
<i>Staphylococcus pseudintermedius</i> DSM-21284	indicator strain	DSMZ
<i>Staphylococcus saprophyticus</i> subsp. <i>saprophyticus</i> DSM-20229	indicator strain	DSMZ
<i>Staphylococcus schleiferi</i> subsp. <i>coagulans</i> DSM-6628	indicator strain	DSMZ
<i>Streptococcus agalactiae</i> IBB123	indicator strain	IBB PAS
<i>Streptococcus agalactiae</i> IBB130	indicator strain	IBB PAS
<i>Streptococcus mitis</i> IBB3449	indicator strain	IBB PAS
<i>Streptococcus sobrinus</i> IBB3450	indicator strain	IBB PAS
<i>Streptococcus parauberis</i> IBB272	indicator strain	IBB PAS
<i>Lactococcus garvieae</i> IBB3403 - spontaneous mutants		
PW202-PW204	GarQ-resistant, indicator strains	12
LGN1, LGN2, LGN4, LGN9	GarQ-resistant, indicator strains	12
MS1011-MS1014, MS1016-MS1018, MS1027-MS1029, MS1031-MS1033, MS1035-MS1036	GarA-resistant	This study
LGA2, LGA3, LGA5, LGA6, LGA13	GarA-resistant	This study
LGB1-LGB10	GarB-resistant	This study
LGC1, LGC3-LGC6, LGC8, LGC9, LGC11-LGC13, LC15, LGC16, LGC18-LGC20	GarC-resistant	This study
<i>Lactococcus garvieae</i> IBB3403 - other mutants		
MS1-MS3	strains after pGhost9::ISS1 integration into chromosome	This study
B548a	strain with <i>manABCD</i> deletion	This study
B549a	strain with <i>manCD</i> deletion	This study
B550a	strain with <i>manC</i> deletion	This study
B551a	strain with <i>manD</i> deletion	This study
<i>Lactococcus garvieae</i> IBB3403 - other strains		
:pGh9::ISS1	strain carrying pGhost9::ISS1	This study
:pGh9: <i>manABCD</i>	strain carrying pGhost9 with <i>manABCD</i> deletion	This study
:pGh9: Δ <i>manCD</i>	strain carrying pGhost9 with <i>manCD</i> deletion	This study
:pGh9: Δ <i>manC</i>	strain carrying pGhost9 with <i>manC</i> deletion	This study
:pGh9: Δ <i>manD</i>	strain carrying pGhost9 with <i>manD</i> deletion	This study
B552a	B548a strain carrying pNZ9530	This study

<i>Lactococcus lactis</i> IL1403 strains with deleted <i>ptnABCD</i>		
B464	strain with <i>ptnABCD</i> deletion	LMBT NMBU ⁴
B488	B464 carrying pNZ9530	LMBT NMBU ⁴
B520	B488 carrying pNZ8037	LMBT NMBU ⁴
B557a	B488 carrying pNZ8037 with <i>manABCD</i>	This study
B558a	B488 carrying pNZ8037 with <i>manCD</i>	This study
B559a	B488 carrying pNZ8037 with <i>manC</i>	This study
B560a	B488 carrying pNZ8037 with <i>manD</i>	This study
B561a	B488 carrying pNZ8037 with <i>manCD</i> without γ^+	This study
<i>Lactococcus lactis</i> NZ9000		
B562a	strain carrying pNZ8037	This study
B563a	strain carrying pNZ8037 with <i>manC</i>	This study
B564a	strain carrying pNZ8037 with <i>manD</i>	This study
<i>Escherichia coli</i> EC1000		
:pGh9::ISS1	strain carrying pGhost9::ISS1	This study
MS1 - MS3	strains carrying pGhost9::ISS1 with part of <i>manABCD</i>	This study
:pGEMT: Δ <i>manABCD</i>	strain carrying pGEMT with <i>man</i> operon deletion	This study
:pGEMT: Δ <i>manCD</i>	strain carrying pGEMT with <i>manCD</i> deletion	This study
:pGEMT: Δ <i>manC</i>	strain carrying pGEMT with <i>manC</i> deletion	This study
:pGEMT: Δ <i>manD</i>	strain carrying pGEMT with <i>manD</i> deletion	This study
:pGh9: Δ <i>manABCD</i>	strain carrying pGhost9 with <i>man</i> operon deletion	This study
:pGh9: Δ <i>manCD</i>	strain carrying pGhost9 with <i>manCD</i> deletion	This study
:pGh9: Δ <i>manC</i>	strain carrying pGhost9 with <i>manC</i> deletion	This study
:pGh9: Δ <i>manD</i>	strain carrying pGhost9 with <i>manD</i> deletion	This study
:pNZ8037: <i>manABCD</i>	strain carrying pNZ8037 with <i>man</i> operon	This study
:pNZ8037: <i>manCD</i>	strain carrying pNZ8037 with <i>manCD</i>	This study
:pNZ8037: <i>manC</i>	strain carrying pNZ8037 with <i>manC</i>	This study
:pNZ8037: <i>manD</i>	strain carrying pNZ8037 with <i>manD</i>	This study
:pNZ8037: <i>manCDA</i> γ^+	strain carrying pNZ8037 with <i>manCD</i> without γ^+	This study
Plasmids		
pGhost9::ISS1	Em ^r , <i>repA</i> (Ts), ISS1	32
pGhost9	Em ^r , <i>repA</i> (Ts)	32
pGEMT	Amp ^r , M13ori, linear T-overhang vector	Promega
pNZ9530	Em ^r , carrying nisin-regulatory <i>nisRK</i> genes	34
pNZ8037	Cam ^r , nisin regulated expression system containing nisin-responsive promoter	33
Primers		
	DNA sequence (5'→3'), <u>restriction site</u>	
pISS1Eco/pGh9	CTAAATAGACTTATCAG/GACAGCTTCCAAGGAGC	
uni/pISS1Hind	GTAACACGACGGCCAGT/AGCTTAAGAACAAGAAGG	
<i>manC</i> for/rev	CGTGATCTCGGCGTTA/TAACGCTCAAGCGTGTG	
<i>manD</i> for/rev	CGCTCTTATCTACCTC/GCCAATTTAGTGCTCCTAAC	
1224/1233	CGCCAGGGTTTTCCCAGTCACGA/AGCGGATAACAATTTACACACAGG	
pGhfor/rev	TGTAAACGACGGCCAGTG/AGTACCGTTACTTATGAGC	
pNZ8037for/rev	CGATAACGCGAGCATA/GCTCAAGGGCTTTTACG	
<i>manABCD</i> UPfor/rev	GCTTTCATGGCCTTATAG/GAGAATTTCGTTGGCTCGCAATAAC	
<i>manABCDDN</i> for/rev	GAGAATTTCACCACTGCTGGTATTG/CGTCGTTATGGGACTA	
<i>manCD</i> UPfor/rev	TGCACGTATCGACTCAC/GAGAATTTCGAAGTCCAGCTAAGAAC	
<i>manCDN</i> for/rev	GAGAATTTCGGCGATATTCTCAAC/GGACCAGTGAAGCTGATG	
<i>manD</i> UPfor/rev	GCCGCTATCTTGTTGG/GAGAATTTCGAACGCTCAAGCGTGTG	
<i>manABCD</i> for/rev	CCGTGGTTTGCTCTAC/TGCTGCTGCGATGAGT	
<i>complman</i> for/rev	ATGCCCATGGATGATCTTCGGTGAGCAAGAG/ATGCCCTCGAGTTATTAAGCA	
	AGACCTGCAACGT	
<i>complCD</i> for	TAGCCCATGGATGAGTATTATTTCATCATTTTATG	
<i>complC</i> rev	TAGCCTCGAGTTAATAATCGTTGAGAATATCG	
<i>complD</i> for	TAGCCCATGGATGGAAAATACAAATAAAAAGTTTAG	
γ^+ for/rev	ACAGTTGATGGTTCTA/TTTATCGATCCATTC	

* Restriction sites are underlined (EcoRI; NcoI; XhoI).

* Bacterial strains derived from the Regional Strains and Plasmids Collection of the Institute of Biochemistry and Biophysics, Warsaw, Poland (IBB PAS), from the Pure Cultures Collection of the Institute of Fermentation Technology and Microbiology, Technical University of Lodz, Poland (LOCK), from the dietary supplement Dicoflor, Vitis Pharma, Poland (Dicoflor), the collection of the Laboratory of Microbial Gene Technology, Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway (LMGT NMBU), the Collection of Microorganisms and Cell Cultures, Germany (DSMZ), the MoBiTec GmbH, Germany (MoBiTec), the collection of the National Institute for Agricultural Research, France (INRA), the American Type Culture Collection (ATCC), and obtained in this study.

Table S2. Inhibitory spectrum of GarA, GarB and GarC. “–” indicates that no inhibition zone was observed, “+” indicates a wide, clear inhibition zone while +/- indicates a minimal, vague inhibition zone observed only at high concentration of bacteriocin (1 mg/ml).

Indicator strain	GarA	GarB	GarC
<i>Bacillus cereus</i> IBB3390	-	-	-
<i>Bacillus subtilis</i> BSB1	-	-	-
<i>Campylobacter jejuni</i> 12	-	-	-
<i>Campylobacter jejuni</i> 480	-	-	-
<i>Campylobacter jejuni</i> 81176	-	-	-
<i>Campylobacter coli</i> 23/1	-	-	-
<i>Candida albicans</i> CAI-4	-	-	-
<i>Carnobacterium maltaromaticum</i> IBB3447	+/-	-	-
<i>Enterococcus durans</i> IBB3441	+/-	-	-
<i>Enterococcus faecalis</i> IBB3439	-	-	-
<i>Enterococcus faecalis</i> IBB3444	-	-	-
<i>Enterococcus faecalis</i> LMGT 2003	-	-	-
<i>Enterococcus faecium</i> LMGT 2783	+/-	-	-
<i>Enterococcus faecium</i> LMGT 2787	+/-	-	-
<i>Lactobacillus casei</i> IBB3418	-	-	-
<i>Lactobacillus casei</i> IBB3427	-	-	-
<i>Lactobacillus casei</i> LOCK 0919	-	-	-
<i>Lactobacillus casei/paracasei</i> IBB3423	-	-	-
<i>Lactobacillus casei/paracasei</i> IBB3425	-	-	-
<i>Lactobacillus casei/paracasei</i> IBB3426	-	-	-
<i>Lactobacillus casei/paracasei</i> IBB3428	-	-	-
<i>Lactobacillus paracasei</i> IBB3424	-	-	-
<i>Lactobacillus johnsonii</i> IBB3155	+/-	-	-
<i>Lactobacillus kunkeei</i> AH1	-	-	-
<i>Lactobacillus kunkeei</i> AH38	-	-	-
<i>Lactobacillus kunkeei</i> AH119	-	-	-
<i>Lactobacillus paraplantarum</i> IBB3438	+/-	-	+/-
<i>Lactobacillus plantarum</i> NC8	-	-	+/-
<i>Lactobacillus plantarum</i> WCSF1	-	-	-
<i>Lactobacillus plantarum</i> IBB3036	-	-	-
<i>Lactobacillus plantarum</i> IBB3433	+/-	-	+/-
<i>Lactobacillus plantarum</i> IBB3436	+/-	-	-
<i>Lactobacillus plantarum</i> subsp. <i>plantarum</i> IBB3434	+/-	-	-
<i>Lactobacillus rhamnosus</i> IBB3429	-	-	-
<i>Lactobacillus rhamnosus</i> LOCK 0900	+/-	-	-
<i>Lactobacillus rhamnosus</i> LOCK 0908	+/-	-	-
<i>Lactobacillus rhamnosus</i> GG	-	-	-
<i>Lactobacillus salivarius</i> IBB3154	+/-	-	+/-
<i>Lactococcus garvieae</i> IBB3403	+	+	+
<i>Lactococcus garvieae</i> IBB66	+	+	+
<i>Lactococcus lactis</i> IBB3404	+/-	-	+
<i>Lactococcus lactis</i> IBB3411	+/-	-	+
<i>Lactococcus lactis</i> NZ9000	+/-	-	+
<i>Lactococcus lactis</i> QU5 LMGT 3419	+/-	-	+
<i>Lactococcus lactis</i> subsp. <i>cremoris</i> IBB3409	+/-	-	+
<i>Lactococcus lactis</i> subsp. <i>lactis</i> IL1403	+/-	-	+
<i>Lactococcus lactis</i> subsp. <i>lactis</i> IBB2955	+/-	-	+
<i>Lactococcus lactis</i> subsp. <i>lactis</i> IBB3407	+/-	-	+
<i>Lactococcus raffinolactis</i> IBB91	-	-	+
<i>Leuconostoc lactis</i> IBB3446	+/-	-	-
<i>Leuconostoc mesenteroides</i> IBB3442	-	-	-
<i>Leuconostoc mesenteroides</i> IBB3443	-	-	+/-
<i>Listeria monocytogenes</i> EGD-e LMGT 2604	-	-	-
<i>Pediococcus acidilacti</i> LMGT 2002	+/-	-	-
<i>Pediococcus parvulus</i> IBB3448	-	-	-
<i>Pediococcus pentosaceus</i> IBB3369	-	-	-
<i>Pseudomonas aeruginosa</i> ATCC 9027	-	-	-
<i>Salmonella typhimurium</i> TT622	-	-	-
<i>Staphylococcus aureus</i> ATCC 6538	-	-	-
<i>Staphylococcus caprae</i> DSM-20608	-	-	-
<i>Staphylococcus delphini</i> DSM-20771	-	-	-

<i>Staphylococcus epidermidis</i> DSM-20044	-	-	-
<i>Staphylococcus hyicus</i> subsp. <i>chromogenes</i> DSM-20454	-	-	-
<i>Staphylococcus intermedius</i> DSM-20373	-	-	-
<i>Staphylococcus lugdunensis</i> DSM-4804	-	-	-
<i>Staphylococcus pseudintermedius</i> DSM-21284	-	-	-
<i>Staphylococcus saprophyticus</i> subsp. <i>saprophyticus</i> DSM-20229	-	-	-
<i>Staphylococcus schleiferi</i> subsp. <i>coagulans</i> DSM-6628	-	-	-
<i>Streptococcus agalactiae</i> IBB123	-	-	-
<i>Streptococcus agalactiae</i> IBB130	-	-	-
<i>Streptococcus mitis</i> IBB3449	-	-	-
<i>Streptococcus sobrinus</i> IBB3450	-	-	-
<i>Streptococcus parauberis</i> IBB272	-	-	-
